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A study on mycotic infections among sputum positive pulmonary tuberculosis patients in Salem district".

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ABSTRACT

Pulmonary tuberculosis is a contagious bacterial infection caused by Mycobacterium tuberculosis. The cavities in the lungs facilitate the growth of fungi by providing plenty of oxygen and necrotic tissue material. Prolonged chemotherapy in these patients also facilitate fungal infection. This study was done to know the prevalence of fungal infections among pulmonary tuberculosis patients and to identify the common fungi causing infection. A total of 107 pulmonary tuberculosis patients were included in the study. Sputum samples were collected and subjected to Gram staining and KOH mount and then cultured on Sabouraud's Dextrose Agar with gentamycin. About 38% of patients had fungal co-infection. The most common fungi were Candida species (18%) followed by Aspergillus species (15%). Most of the times these fungal infections are not diagnosed and often mistaken for recurrence of tuberculosis. Hence adequate measure must be taken for the early diagnosis and treatment of these opportunistic infections, which are associated with high rate of morbidity and mortality.

KEYWORDS Pulmonary tuberculosis, Opportunistic infections, Aspergillus species, Candida species.

Introduction:

Pulmonary tuberculosis is a contagious bacterial infection caused by *Mycobacterium tuberculosis*. It ranks as the second leading cause of death due to an infectious disease worldwide next to Human Immunodeficiency Virus (1). About one- third of the world's population is latently infected with *Mycobacterium tuberculosis* and over 8 million new cases are reported every year (2). About one-third of patients with active tuberculosis die within a year and half die within 5 years if left untreated. The World Health Organization (WHO) declared tuberculosis a global public health emergency in 1993 (1). The consequences of infection with *Mycobacterium tuberculosis* depend mainly upon the immunocompetence of the host. After initial lung infection, *M. tuberculosis* may spread to regional lymph nodes and then throughout the body depending on the immunity of the host.

Pulmonary tuberculosis is a chronic destructive lung disease with caseation, necrosis and fibrosis that lead to the formation of cavities. These cavities facilitate the growth of many organisms including fungi by providing plenty of oxygen and necrotic tissue material. Inaddition, prolonged chemotherapy in these patients also facilitate fungal infection (3). Mainly four types of fungi, i.e. *Aspergillus niger, Aspergillus fumigatus, Histoplasma capsulatum* and *Cryptococcus neoformans* are isolated from tuberculosis patients.The commonest among these is *Aspergillus species* (4). If aspergillosis begins to spread rapidly through the lungs, it can cause cough, fever, chest pain and difficulty in breathing that doesn't respond to antibiotics which could be fatal. Candida species are also emerging as potentially pathogenic fungal agent in patients with broncho-pulmonary disease (5). Active mycosis though an independent marker of advanced immunosuppression, may also act as a co-factor responsible for rapid progression of the disease (4). Most of the times these fungal infections are not diagnosed and often mistaken for recurrence of tuberculosis (6). These opportunistic infections if diagnosed early can be treated effectively to prevent the progression of disease (7).

Aim:

This study was done to know the prevalence of fungal infections among pulmonary tuberculosis patients in Salem district and to identify the common fungi causing infection in these patients.

Materials and Methods:

A total of 107 sputum positive pulmonary tuberculosis patients, both new and old cases attending tuberculosis unit in Salem district were included in the study. The study was conducted in Vinayaka Mission's Kirupananda Variyar Medical College, Salem after obtaining informed consent from the patients and instituitional ethical committee clearance. Both Category I and Category II patients were included in our study. Out of 107 patients, 74 were in Category I and 32 patients were in Category II and one patient was XDR-TB. Sputum samples were collected from these patients and they were subjected to Gram staining and KOH mount. They were then cultured on two tubes of Sabouraud's Dextrose Agar (SDA) with gentamycin and incubated at 37°C and 22°C. A detailed history regarding smoking, alcohol consumption, calorie intake etc., was collected from the patients by administering a questionnaire. The growth on SDA along with hyphal elements in KOH was considered significant. The growth was identified by Lactophenol Cotton Blue staining. Pulmonary candidiasis was diagnosed based on the presence of budding yeast cells along with pseudohy-

phae in Gram staining and creamy colonies on SDA and speciated using CHROMagar.

The fungal growth was identified based on the following findings (8):

Aspergillus fumigatus:
Macroscopic appearance on SDA:
Obverse- velvet or powdery at first turning into smoky green colonies and Reverse- white to tan

Microscopic appearances in LPCB mount:
Hyaline, sepate hyphae with smooth Conidiophore. Uniseriate sterigmata covering the upper half of the vesicle.

Aspergillus niger:
Macroscopic appearance on SDA:
Obverse- wooly at first, white to yellow then turning dark brown to black and Reverse- white to yellow.

Microscopic appearances in LPCB mount:
Hyaline, septate hyphae and Conidiophore are of variable length. Biseriate sterigmata covering the entire vesicle forming a radiate head.

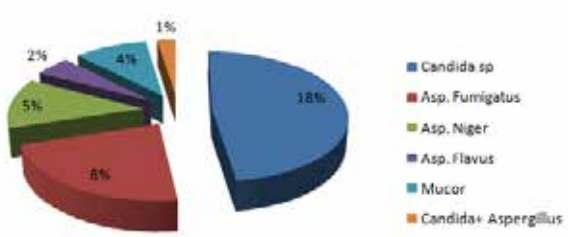
Aspergillus flavus:
Macroscopic appearance on SDA:
Obverse: Velvety, yellow to green powdery colonies and Reverse: Golden to red brown

Microscopic appearances in LPCB mount:
Hyaline, septate hyphae with rough, pitted and spiny conidiophore. Uniseriate or biseriate sterigmata covering the entire vesicle, protruding in all directions.

Identification of Candida:
After inoculation onto CHROMagar, the plates were incubated for 48 hours at 37°C and the colonies were identified based on the color produced by the *Candida* species. Light green colonies- *Candida albicans*, blue colonies with pink halo - *Candida tropicalis*, purple colonies- *Candida glabrata*, pink colonies – *Candida krusei*, cream colonies- *Candida parapsilosis.*

Results:
Fungal co-infection was seen in 38% of pulmonary tuberculosis patients in our study. The common fungi isolated were *Candida species* (18%) followed by *Aspergillus species* (15%). *Candida* coinfection was observed in 19 patients (17.7%). Among these 19 samples, two samples showed dual infection (with two different species of Candida in same sample). Hence total number of *Candida* isolated was 21.

Fig 1: Distribution of fungal infections among the study population (n=107)



About 38% of patients had fungal growth and the most common fungi being Candida species, followed by Aspergillus species.

Table 1: Species distribution of Candida (n=21)

S.No	Species of Candida	No of isolates
1	Candida albicans	14(66.7%)
2	Candida tropicalis	2(9.5%)
3	Candida krusei	2(9.5%)
4	Candida parapsilosis	2(9.5%)
5	Candida glabrata	1(4.8%)

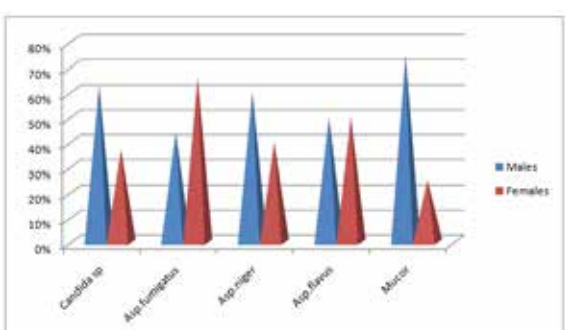
C.albicans was the predominant species causing co-infection among pulmonary tuberculosis patients in our study.

Table 2: Distribution of fungal infections based on the category of DOTS

Fungi	DOTS category			Total
	Category 1(74)	Category 2(32)	XDR TB(1)	
Candida species	12	8	1	21
Aspergillus fumigatus	4	5	0	9
Aspergillus niger	2	3	0	5
Aspergillus flavus	1	1	0	2
Mucor	2	2	0	4
Candida + Aspergillus	0	1	0	1
Total	21/74(28.37%)	20/32(62.5%)	1/1(100%)	42

Fungal infections were more common in Category 2 patients than category 1.

Fig 2: Distribution of fungal infections based on gender



There was no gender specific prevalence of fungal infections among TB patients in our study.

Table 3: Distribution of fungal infections among smokers and non smokers

Fungal infection	Smoking		Total
	Smokers	Non-smokers	
Candida species	14	7	21
Aspergillus fumigatus	7	2	9
Aspergillus niger	2	3	5
Aspergillus flavus	2	0	2
Mucor	3	1	4
Candida + Aspergillus	1	0	1
Total	29(69%)	13(31%)	42

The prevalence of fungal infection was more among smokers when compared to non smokers and this was found to be statistically significant (P < 0.05).

Table 4: Distribution of fungal infections among alcoholics and non alcoholics

Fungal infection	Alcohol		Total
	Alcoholics	Non alcoholics	
Candida species	11	10	21
Aspergillus fumigatus	6	3	9
Aspergillus niger	2	3	5
Aspergillus flavus	1	1	2
Mucor	2	2	4
Candida + Aspergillus	0	1	1
Total	22(52%)	20(46%)	42

The prevalence of fungal infections among alcoholics and non alcoholics did not show any statistically significant difference (P >0.05).

Table 5: Distribution of fungal infections based on BMI

Fungal infection	BMI				Total
	<15	15-18	18.1-21	21.1-24	
Candida species	2	13	4	2	21
Aspergillus fumigatus	2	5	1	1	9
Aspergillus niger	0	3	2	0	5
Aspergillus flavus	1	0	0	1	2
Mucor	1	2	0	1	4
Candida and Aspergillus	1	0	0	0	1
Total	7(17%)	23(55%)	7(17%)	5(12%)	42

The prevalence of fungal infection seems to be more common in patients having low BMI when compared with normal or high BMI and this difference was found to be statistically significant (P<0.0001).

Discussion:

Fungal infections of lungs are the commonest opportunistic infection being encountered in pulmonary tuberculosis patients (4). Each year, more than one million people who have been treated successfully for tuberculosis develop superadded fungal infections and are often mistaken for recurrence of tuberculosis (6).

In our study, about 38% of pulmonary tuberculosis patients were co-infected with fungal agents. This is in concordance with the study conducted by Khanna et al and Bansal et al (3) where 36.36 % and 39.4% of pulmonary tuberculosis patients were coinfected with fungal agents. But ours was in contrast to the study conducted by Shome et al (9) where coinfection was seen only in 18% cases. This difference could be due to the difference in the samples collected. Sputum was collected in our study but bronchial aspirates and bronchoscopic materials were collected in the study by Shome et al (9) which are far better than sputum.

The present study shows 18% of pulmonary tuberculosis patients to be co-infected with *Candida spp*. This is in accordance with the study done by Sehar Afshan Naz and Perween Tariq (10) in which 15.2% of co-infection with *Candida* species was documented. But this is low when compared to the study of VP Baradkar et al (11) which shows a prevalence of 26% co-infection with *Candida* species. Candida forms a part of normal microbial flora of healthy individuals. When the host resistance is lowered, these commensals turn into aggressive pathogens causing life threatening systemic infections.The role of Candida species as secondary invaders in patients having pre-existing diseases like pulmonary tuberculosis is well documented (12).

Among the *Candida spp*, *C. albicans* was the commonest organism causing secondary infection in our study. *C. albicans* constituted 66.7% of the total Candida isolates. This correlates with the study conducted by Kali A et al (5) which also demonstrates *C.albicans* to be the commonest species causing secondary infection comprising 50% of the total *Candida* isolates and Khanna et al where 62% of the *Candida species* isolated comprised of *Candida albicans.*

Among *non-albicans Candida* species, *C. tropicalis* has been emerging as the new opportunistic pathogen causing infection in patients with pre-existing lung disease. *C. tropicalis* has an apparently greater capacity than *C.albicans* to invade the deep tissues of immunocompromised host (10). Most of the *non albicans Candida* exhibit reduced susceptibility to fluconazole (13). Hence identification of *Candida* to the species level becomes mandatory for selecting the appropriate antifungal agents in treatment of invasive candidiasis.

Aspergillus species was isolated from 15% of cases in our study. Our finding is in accordance with the study conducted by Khanna et al (3) where *Aspergillus species* was isolated from 10% and also with Anna N. Njula et al (12) in which the isolation rate of *Aspergillus species* was 15%. But our study is in contrast to that conducted by Sobti et al[3] with an isolation of *Aspergillus species* in 40% cases.

The predominant *Aspergillus species* isolated in our study was *Aspergillus fumigatus* (8.4%), followed by *Aspergillus niger* (4.6%) and *Aspergillus flavus* (1.8%). These rates are much lower than those obtained by Razmpa et al (14) with high rates of 30% of *Aspergillus flavus*. These wide variations in the incidence and isolation of various species of fungi could be related to the geographical differences (11).

There was a statistically significant relationship between smoking and low BMI among male patients with fungal co-infection. In female patients, it was low BMI which was found to be statistically significant. Hence smoking and low BMI could be the factors determining the prevalence of fungal infections among patients with pulmonary tuberculosis.

Conclusion:

The prevalence of opportunistic fungal infection in pulmonary tuberculosis patients cannot be under-estimated. These secondary fungal infections are associated with persistence of lung symptoms inspite of successful completion of antituberculous drug therapy. Hence adequate measures need to be taken for the early identification and treatment of these opportunistic infections, which are associated with high rates of morbidity and mortality.

Inaddition, identification to the species level becomes mandatory in selecting the appropriate antifungal agents.

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